

Claims

1. A method for extracting nucleic acid from a sample containing nucleic acid, which method comprises:

5 at a first pH, bringing the sample into contact with a material which comprises an ionisable group, wherein the material has a positive charge at said first pH, such that nucleic acid is bound to the material; and

10 releasing the nucleic acid at a second, higher, pH at which the charge on the material is negative, neutral or less positive,

wherein the release of the nucleic acid occurs under mild conditions.

15 2. A method according to claim 1, wherein the mild conditions are conditions at which said nucleic acid is not denatured and/or not degraded and/or not depurinated and/or substantially physiological conditions.

20 3. A method according to claim 1, wherein the releasing step occurs at a pH of no more than about 10.5, preferably no more than about 9.0.

25 4. A method according to claim 1, wherein the releasing step occurs at an ionic strength of no more than about 500mM, preferably no more than about 100mM.

5. A method according to claim 1, wherein the releasing step occurs at a temperature of no more than about 70°C, preferably no more than about 50°C.

6. A method according to claim 5, wherein the releasing step occurs at about room temperature.

5 7. A method according to claim 1, wherein the releasing step comprises contacting the bound nucleic acid with a buffer solution to release the nucleic acid, the buffer solution being suitable for the storage or further processing of the released nucleic acid.

10 8. A method according to claim 7, wherein the buffer solution is a buffer solution suitable for PCR.

15 9. A method according to claim 1, wherein the pKa of said ionisable group is between about 3.0 and 9.0, preferably between about 4.0 and 9.0.

20 10. A method according to claim 9, wherein the material comprises a positively ionisable group, the pKa of which is between about 5.0 and 8.0, preferably between about 6.0 and 7.0.

11. A method according to claim 10, wherein the material comprises a weak base.

25 12. A method according to claim 10, wherein the material comprises a biological buffer.

30 13. A method according to claim 10, wherein the material comprises a positively ionisable nitrogen atom and one or more electronegative groups capable of lowering the pKa of the positively ionisable nitrogen atom.

14. A method according to claim 10, wherein the material comprises a chemical species selected from the group consisting of:

- 5 N-2-acetamido-2-aminoethanesulfonic acid (ACES);
 N-2-acetamido-2-iminodiacetic acid (ADA);
 amino methyl propanediol (AMP);
 3-1,1-dimethyl-2-hydroxyethylamino-2-hydroxy
propanesulfonic acid (AMPSO);
- 10 N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid
 (BES);
 N,N-bis(2-hydroxyethyl)glycine (BICINE);
 bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane
 (Bis-Tris);
- 15 1,3-bis(tris(hydroxymethyl)aminopropyl)propane (Bis-Tris
Propane);
 4-cyclohexylamino-1-butane sulfonic acid (CABS);
 3-cyclohexylamino-1-propane sulfonic acid (CAPS);
 3-cyclohexylamino-2-hydroxy-1-propane sulfonic acid
20 (CAPSO);
 2-N-cyclohexylaminoethanesulfonic acid (CHES);
 3-N,N-bis(2-hydroxyethylamino)-2-
hydroxypropanesulfonic acid (DIPSO);
 N-2-hydroxyethylpiperazine-N'-3-propanesulfonic acid
25 (EPPS);
 N-2-hydroxyethylpiperazine-N'-4-butanesulfonic acid
 (HEPBS);
 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
 (HEPES);
- 30 N-2-hydroxyethylpiperazine-N'-2-propanesulfonic acid
 (HEPPSO);

2-N-morpholinoethanesulfonic acid (MES);
4-N-morpholinobutanesulfonic acid (MOBS);
3-N-morpholinopropanesulfonic acid (MOPS);
3-N-morpholino-2-hydroxypropanesulfonic acid
5 (MOPSO);
piperazine-N-N-bis-2-ethanesulfonic acid (PIPES);
piperazine-N-N-bis-2-hydroxypropanesulfonic acid
(POPSO);
N-trishydroxymethyl-methyl-4-aminobutanesulfonic
10 acid (TABS);
N-trishydroxymethyl-methyl-3-aminopropanesulfonic
acid (TAPS);
3-N-trishydroxymethyl-methylamino-2-
hydroxypropanesulfonic acid (TAPSO);
15 N-trishydroxymethyl-methyl-2-aminoethanesulfonic
acid (TES);
N-trishydroxymethylmethylglycine (TRICINE);
trishydroxymethylaminomethane (Tris);
polyhydroxylated amines;
20 histidine, and polyhistidine;
imidazole, and derivatives thereof (i.e.
imidazoles), especially derivatives containing hydroxyl
groups;
triethanolamine dimers and polymers; and
25 di/tri/oligo amino acids, for example; Ala-Ala; Gly-
Gly, pKa 8.2; Ser-Ser; Gly-Gly-Gly, Ser-Gly;
a detergent, such as decylmethylimidazole or
dodecyl-Bis-Tris;
a carbohydrate containing nitrogen and
30 electronegative groups, such as a glucosamine, a

polyglucosamine (e.g. a chitosan), a kanamycin or derivative thereof;

a nucleic acid base, such as cytidine; and

a monomeric, oligomeric or polymeric compound

5 containing an aliphatic or aromatic nitrogen-containing heterocyclic ring, such as morpholine-, pyrrole-, pyrrolidine-, pyridine-, pyridinol-, pyridone-, pyrroline-, pyrazole-, pyridazine-, pyrazine-, piperidone-, piperidine-, or piperazine-containing
10 compounds, e.g. polyvinylpyridine, said ring optionally being substituted with one or more electronegative groups.

15 15. A method according to claim 14, wherein the chemical species is selected from the group consisting of:

Tris;

Bis-Tris;

Bis-Tris Propane;

Tricine;

20 Bicine;

polyhydroxylated amines; and

polyhistidine.

25 16. A method according to claim 9, wherein the material comprises:

a negatively ionisable group, the pKa of which is between about 3.0 and 7.0;

and a group which is positively charged at said first pH, and optionally also at said second pH.

30

17. A method according to claim 16 wherein said negatively ionisable group is a carboxy group.

18. A method according to claim 16 wherein said group
5 which is positively charged is a metal oxide, such as iron II,III oxide.

19. A method according to claim 1, wherein the material
10 comprises an ionisable group having a pKa value, said pKa value being between the first and second pH, or within about 1.0 pH unit, preferably within about 0.5 pH unit, below said first pH.

20. A method according to claim 19, wherein said second
15 pH is within about 3 pH units, preferably within about 2 pH units, above the pKa value.

21. A method according to claim 1, wherein the method is
20 for separating single stranded nucleic acid from double stranded nucleic acid.

22. A method according to claim 1, wherein the method is
25 for extracting single stranded nucleic acid, said method comprising a prior step of converting double stranded nucleic acid into single stranded nucleic acid.

23. A method according to claim 1, wherein the material is a solid phase material.

24. A method according to claim 1, wherein the binding step occurs in a solution having a concentration of 1M or less.

5 25. A solid phase product for use in a method of extracting nucleic acid from a sample, the product comprising a plurality of positively ionisable groups, the ionisable groups being provided by a chemical species selected from the list consisting of:

10 biological buffers;
polyhydroxylated amines;
histidine; and
polyhistidine.

26. A product according to claim 25 wherein the biological buffer is selected from the group consisting of:

5
SUB A2
15
20
25
30
N-2-acetamido-2-aminoethanesulfonic acid (ACES);
N-2-acetamido-2-iminodiacetic acid (ADA);
amino methyl propanediol (AMP);
3-1,1-dimethyl-2-hydroxyethylamino-2-hydroxy
propanesulfonic acid (AMPSO);
N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid
(BES);
N,N-bis(2-hydroxyethyl)glycine (BICINE);
bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane
(Bis-Tris);
1,3-bis(tris(hydroxymethyl)methylaminopropane (Bis-Tris
Propane);
4-cyclohexylamino-1-butane sulfonic acid (CABS);
3-cyclohexylamino-1-propane sulfonic acid (CAPS);

- 3-cyclohexylamino-2-hydroxy-1-propane sulfonic acid
(CAPSO);
2-N-cyclohexylaminoethanesulfonic acid (CHES);
3-N,N-bis-2-hydroxyethylamino-2-
5 hydroxypropanesulfonic acid (DIPSO);
N-2-hydroxyethylpiperazine-N-3-propanesulfonic acid
(EPPS);
N-2-hydroxyethylpiperazine-N-4-butanesulfonic acid
(HEPBS);
10 N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
(HEPES);
N-2-hydroxyethylpiperazine-N-2-propanesulfonic acid
(HEPPSO);
2-N-morpholinoethanesulfonic acid (MES);
4-N-morpholinobutanesulfonic acid (MOBS);
3-N-morpholinopropanesulfonic acid (MOPS);
3-N-morpholino-2-hydroxypropanesulfonic acid
(MOPSO);
piperazine-N,N-bis-2-ethanesulfonic acid (PIPES);
20 piperazine-N,N-bis-2-hydroxypropanesulfonic acid
(POPSO);
N-trishydroxymethyl-methyl-4-aminobutanesulfonic
acid (TABS);
N-trishydroxymethyl-methyl-3-aminopropanesulfonic
25 acid (TAPS);
3-N-trishydroxymethyl-methylamino-2-
hydroxypropanesulfonic acid (TAPSO);
N-trishydroxymethyl-methyl-2-aminoethanesulfonic
acid (TES);
30 N-trishydroxymethylmethylglycine (TRICINE);
trishydroxymethylaminomethane (Tris);

SUB A2
C-14

polyhistidine;
polyhydroxylated imidazoles;
triethanolamine dimers and polymers; and
di/tri/oligo amino acids, for example Gly-Gly, Ser-
5 Ser, Gly-Gly-Gly, and Ser-Gly.

27. A product according to claim 25, wherein the
plurality of ionisable groups are separately immobilised
on a solid support by covalent or ionic bonding or by
10 adsorption.

28. A product according to claim 25, wherein the
plurality of ionisable groups are separately attached to
a polymer, said polymer being immobilised on a solid
15 support by covalent or ionic bonding or by adsorption.

29. A product according to claim 25, wherein the
ionisable groups are polymerised, optionally by means of
cross-linking reagents.
20

30. A product according to claim 29, wherein the polymer
is immobilised on a solid support by covalent or ionic
bonding or by adsorption.

25 31. A product according to claim 29, wherein the polymer
is a solid.

32. A product according to claim 29 which is a
container.
30

33. A container according to claim 32 which is a PCR or storage tube or well, or a pipette tip.

34. A water soluble product for use in a method of extracting nucleic acid from a sample, the product comprising a plurality of positively ionisable groups, the ionisable groups being provided by a chemical species selected from the list consisting of:

biological buffers;
polyhydroxylated amines;
histidine; and
polyhistidine.

35. A product according to claim 34 wherein the biological buffer is selected from the group consisting of:

N-2-acetamido-2-aminoethanesulfonic acid (ACES);
N-2-acetamido-2-iminodiacetic acid (ADA);
amino methyl propanediol (AMP);
3-1,1-dimethyl-2-hydroxyethylamino-2-hydroxy
propanesulfonic acid (AMPSO);
N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid
(BES);
N,N-bis(2-hydroxyethyl)glycine (BICINE);
bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane
(Bis-Tris);
1,3-bis(tris(hydroxymethyl)methylaminopropane (Bis-Tris
Propane);
4-cyclohexylamino-1-butane sulfonic acid (CABS);
3-cyclohexylamino-1-propane sulfonic acid (CAPS);

SUB A3

3-cyclohexylamino-2-hydroxy-1-propane sulfonic acid
(CAPSO);
2-N-cyclohexylaminoethanesulfonic acid (CHES);
3-N,N-bis-2-hydroxyethylamino-2-
5 hydroxypropanesulfonic acid (DIPSO);
N-2-hydroxyethylpiperazine-N-3-propanesulfonic acid
(EPPS);
N-2-hydroxyethylpiperazine-N-4-butanesulfonic acid
(HEPBS);
10 N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
(HEPES);
N-2-hydroxyethylpiperazine-N-2-propanesulfonic acid
(HEPPSO);
2-N-morpholinoethanesulfonic acid (MES);
15 4-N-morpholinobutanesulfonic acid (MOBS);
3-N-morpholinopropanesulfonic acid (MOPS);
3-N-morpholino-2-hydroxypropanesulfonic acid
(MOPSO);
piperazine-N-N-bis-2-ethanesulfonic acid (PIPES);
20 piperazine-N-N-bis-2-hydroxypropanesulfonic acid
(POPSO);
N-trishydroxymethyl-methyl-4-aminobutanesulfonic
acid (TABS);
N-trishydroxymethyl-methyl-3-aminopropanesulfonic
25 acid (TAPS);
3-N-trishydroxymethyl-methylamino-2-
hydroxypropanesulfonic acid (TAPSO);
N-trishydroxymethyl-methyl-2-aminoethanesulfonic
acid (TES);
30 N-trishydroxymethylmethylglycine (TRICINE);
trishydroxymethylaminomethane (Tris);

polyhistidine;
polyhydroxylated imidazoles;
triethanolamine dimers and polymers; and
di/tri/oligo amino acids, for example Gly-Gly, Ser-
5 Ser, Gly-Gly-Gly, and Ser-Gly.

36. A product according to claim 34, wherein the
plurality of ionisable groups are separately attached to
a polymer.
10

37. A product according to claim 34, wherein the
ionisable groups are polymerised, optionally by means of
cross-linking reagents.

38. A product for use in a method of extracting nucleic
acid from a sample, wherein the product possesses a
positive charge at both a first pH at which it is desired
to bind nucleic acid and a second higher pH at which it
is desired to release nucleic acid, the product
15 comprising a plurality of negatively ionisable groups,
the combined charge of which becomes more negative
between said first pH and said second pH, such that the
product is capable of binding nucleic acid at said first
pH, which bound nucleic acid is released from the product
20 at said second pH.
25

39. A product according to claim 38, wherein the
negatively ionisable group has a pKa between about 3 and
7, preferably between about 4 and 7.
30

40. A product according to claim 38, wherein the negatively ionisable is a carboxy group.

41. A product according to claim 38 wherein said
5 positive charge is provided by a metal or metal oxide,
preferably iron II,III oxide.

ADD A4)

[illegible]